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Bioturbation transports secondary microplastics to deeper layers in soft marine sediments of the northern Baltic Sea

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Abstract

Microplastics (MPs) are observed to be present on the seafloor ranging from coastal areas to deep seas. Because bioturbation alters the distribution of natural particles on inhabited soft bottoms, a mesocosm experiment with common benthic invertebrates was conducted to study their effect on the distribution of secondary MPs (different-sized pieces of fishing line <1 mm). During the study period of three weeks, the benthic community increased MP concentration in the depth of 1.7–5.1 cm in the sediment. The experiment revealed a clear vertical gradient in MP distribution with their abundance being highest in the uppermost parts of the sediment and decreasing with depth. The Baltic clam *Macoma balthica* was the only study animal that ingested MPs. This study highlights the need to further examine the vertical distribution of MPs in natural sediments to reliably assess their abundance on the seafloor as well as their potential impacts on benthic communities.

Key words: microplastic, ingestion, *Macoma balthica*, *Monoporeia affinis*, *Marenzelleria* spp.

1. Introduction

Extensive production of plastics started in the middle of the 20th century and has been growing ever since (PlasticsEurope, 2013). The wide usage of plastics and poor management practices have resulted in the accumulation of plastic litter in the oceans. Plastics in general are long-lived, and thus persistent in the marine environment (Andrady, 2015). In addition to the concern about macro-sized plastic pollution around the globe, focus has recently shifted towards microplastics (hereafter MPs), which are generally defined as small (<5 mm) plastic particles that have either been intentionally manufactured to be small (primary MPs) or have fragmented from larger plastic items (secondary MPs) (UNEP, 2016).

MPs have been observed everywhere in the oceans including surface waters (Eriksen et al., 2013; Setälä et al., 2016a), the water column (Kukulka et al., 2012; Reisser et al., 2015), sea ice (Obbard et al., 2014) and the seafloor (Claessens et al., 2011; Van Cauwenberghe et al., 2013). Due to their small size and ubiquitous distribution, MPs may be potentially harmful to marine biota because they can be ingested by a variety of both pelagic and benthic species (Murray and Cowie, 2011; Lusher et al., 2013; Van Cauwenberghe et al., 2015).

The special characteristics of plastic polymers affect their distribution in the sea. Typically plastics less dense than seawater (1.025 g/cm^3), such as common consumer plastics polyethylene and polypropylene, tend to float on the sea surface whereas denser plastic types are suspended in the water column or sink to the seafloor (Andrady, 2011). However, items made of less dense plastic polymers can also eventually sink as a result of biofilm formation (Lobelle and Cunliffe, 2011), after being ingested and subsequently egested in faecal pellets (Cole et al., 2013), or being convoyed with phytoplankton aggregates (Long et al., 2015). Environmental sampling has

confirmed that MPs found in or on the seafloor include plastic types that are typically positively buoyant in seawater (Claessens et al., 2011; Vianello et al., 2013). Therefore, the seafloor is proposed to serve as an ultimate sink for marine MPs (Woodall et al., 2014).

Fine-grained soft sediments make up most of the seafloor (Rhoads, 1974), but there is currently little information on the fate of MPs when they reach these habitats. In colonized soft bottoms, animals alter their habitats by influencing the sediment structure in a process called bioturbation (Kristensen et al., 2012). Bioturbation covers all the actions of benthic fauna, such as burrowing, ingestion, defecation and ventilation, that directly or indirectly transport particles or solutes in the sediment matrix (Kristensen et al., 2012). As bioturbation is known to increase the surface area available for particle-exchange between sediment and overlying water (Karlson et al., 2007), we hypothesized that it would also affect the transport of MPs in the sediments. A mesocosm experiment was therefore established in order to investigate how the bioturbation caused by invertebrates in the soft bottom sediments would shape the vertical distribution of secondary MPs on the seafloor.

2. Material and methods

2.1. Sample collection and experimental set up

The experiment was conducted at Tvärminne Zoological Station (University of Helsinki), southwest Finland, northern Baltic Sea. The sediment and the animals for the experiment were collected close to the station aboard R/V Saduria in February and April 2015 with a van Veen grab at three locations (N59°51′09″ E23°15′25″, depth 7 m; N59°51′16″ E23°15′25″, depth 20 m; N59°51′32″

E23°15′82″, depth 34 m) and with a bottom trawl at one location (N59°51′18″ E23°16′23″, depth 36 m).

The collected sediment was sieved through 1 mm sieve to remove all animals. Sieved sediment from different sampling sites was mixed together to generate a large amount of homogenous sediment, which was then divided into 30 cylinders (height 20cm, diameter 14 cm) with a movable bottom (Viitasalo-Frösén et al., 2009) and placed in a temperature-controlled room (10 °C). The cylinders were covered with 500 μm steel mesh lids and a hose was placed horizontally above every set of five units. Small holes drilled to the hose allowed a continuous and gentle dropping of ambient seawater (salinity 5–6, temperature 5 °C, oxygen 11.6 mg/L at the start of the acclimatization period) to the units. The sediment in the cylinders was left overnight to settle. The next day 16 individuals of *M. balthica* (mean size 17.3 cm) were added to each of 15 units, and left to acclimatize for 9 weeks. The remaining 15 units served as controls with no animals. During the acclimatization period food was added to all units twice a week (Shellfish diet 1800, Reef Mariculture): feeding was terminated one week prior to the start of the experiment. Polychaete worms (*Marenzelleria* spp.) and amphipods (*Monoporeia affinis*) were collected in April and added to the units containing *M. balthica* one day prior to starting the experiment. The abundances of all the benthic animals used in the experiment were adjusted close to natural densities found in the northern Baltic Sea (Table 1).

Secondary MPs were produced by cutting fishing line (Trilene sensation, Berkley) with a McIlwain™ Tissue Chopper. The diameter of the fishing line was approx. 200 μm and it was cut into three different lengths: 50, 150 and 300 μm. Each size class was cut from a different coloured fishing line, weighted and divided into 30 separate portions using a Mettler Toledo XS205 Dual Range scale. The scale was also used to estimate the concentration of MP additions. Additions to

each unit were approximately 490 pieces (50 µm), 880 pieces (150 µm) and 390 pieces (300 µm), which correspond to a concentration of 114 400 pieces/m^2, 880 pieces/L of sediment, 1 790 pieces/kg of dry sediment. Relatively high concentration of MPs was used in case there would be problems with their extraction from the sediment. The experiment started when MPs were added to the units. When starting the experiment the water temperature was 6 °C and oxygen content 10.8 mg/L (YSI Environmental ProODO™).

After the experiment had been running for a week, 10 units (5 control units, 5 animal units) were randomly selected and terminated. Sediment from the units was sliced to six layers according to depth (approx. 1.7 cm per slice). The cylinder was lifted on the slicing device (HAPS corer sample ejection aggregate) and a cutting plate was attached on top of the cylinder. When rotating the piston of the device, the sediment in the cylinder was pushed upwards allowing the cutting plate to slice the sediment. The sediment slices were then sealed in ziplock bags and frozen in –20 ° C. The rest of the units were terminated and handled in similar manner after two and three weeks.

2.2. Microplastics extraction and sample processing

Frozen sediment slices were thawed at room temperature and animals were handpicked and preserved in 70% ethanol. MPs were extracted from the sediment samples using saturated salt solution (Thompson et al., 2004). The original method was modified by adding solid NaCl to the wet sediment sample according to its volume and maximum solubility (35.7 g NaCl/100 ml sample) to compensate dilution of the solution due to the high water content of the sediment sample. The sample was then mixed and salt allowed to dissolve for 20 minutes before further processing. This solid NaCl addition raised the density of the salt solution during the first extraction step; however, if

124 solid NaCl is used in processing environmental samples, great care must be taken because the salt
125 can act as an additional source of MP contamination (P.N., personal observation).
126
127 Saturated NaCl solution was added until the total volume of the sample was one litre. The sample
128 was stirred for one minute and allowed to settle for 8 minutes. Cleared supernatant was suctioned
129 with a hose through a 100 µm plankton net filter. The small residue above the sediment surface was
130 decanted on a separate 100 µm plankton net filter because it has been observed that most of the
131 MPs are retrieved in the decanting phase (Stolte et al., 2015). These phases were repeated twice
132 without additional solid NaCl to ensure the best possible yield of MPs (Browne et al., 2011;
133 Claessens et al., 2011; Martins and Sobral, 2011). The MPs caught on filters were examined using a
134 stereomicroscope (Leica CLS 150 XE, Schott KL 1500, 0.63–5.0× magnification). Extraction
135 efficiency calculated from the samples was 49.5% (excluding MPs ingested by study animals). The
136 extraction efficiency was better for bigger particles (300 µm: 83.4%. 150 µm: 43.1%, 50 µm:
137 34.3%). Most of the extracted MPs (62.2%) were retrieved during the first extraction step; the
138 second extraction step yielded an additional 21.7% of particles and the third 16.1%. Decanting
139 proved to be more efficient compared to suction with a hose in every extraction step; altogether
140 64% of all the microplastics were recovered when decanting the supernatant residue after suction
141 with a hose.
142
143 Grain size analysis was performed separately for all sediment layers of one control unit for
144 background information. Prior to the analysis the salt residue from the density separation was
145 washed away by mixing 1700 ml of pure H_2O with the dried sediment sample and waiting 2 h for
146 the salt residue to dissolve in the water. The supernatant was then removed with a hose and phases
147 repeated once more. Each sample was covered with 6% H_2O_2 for two days and stirred twice a day
148 to digest all organic material in the sediment. Samples were sieved wet through 500, 250 and 63 µm

sieves. The material from the sieves was washed into pre-weighted containers and dried at 60 °C to determine the dry weight of each size fraction. Water and the <63 µm size fraction that passed the smallest sieve was left to settle for two days. The water was then sucked with a hose without disturbing the sediment at the bottom and the sediment was then washed into a pre-weighted container and dried at 60 °C before weighting.

To count the ingested MPs, 75 individual *M. balthica* (5 clams from each unit) and 57 individual *Marenzelleria* spp. (3–5 polychaetes from each unit) were dissected. Because at the end of the experiment *M. affinis* was not retrieved from all the units, 2 individuals from each of 12 units containing them were examined. All the animals were rinsed in a jar containing pure tap water to wash away dirt and microplastics that could have been attached on their surfaces. Individual *M. balthica* were measured and rinsed again after being detached from their shells with a scalpel. The mantle and foot were removed, the gills separated to an object glass and the remainder of the animal tissue was evenly distributed and placed in an Utermöhl settling chamber. Individual *M. affinis* were placed on their sides on separate object glasses, their carapace opened from the back and the digestive tract pulled out. The rest of the animal was inspected on the same object glass. *Marenzelleria* individuals could not be measured because they had broken into pieces while picking them from the sediment. Individuals from each layer were pooled as one sample and placed on one object glass. All the animals were dissected under a stereomicroscope (Leica CLS 150 XE, Schott KL 1500, 0.63–5.0× magnification) and inspected with an epifluorescence microscope (Leica DMI 3000 B, Leica I3 filter cube, 0.4–40× magnification), because the fishing lines were fluorescent under blue light (excitation BP 450–490).

2.3. Statistical analysis

Because the exact density of used fishing line was not known and some particles were found to get stuck on the water surface of the units, there was a strong possibility that different numbers of MPs ended up sinking into the sediment. Therefore, we decided to examine the percentages of found MPs instead of actual numbers added to each unit. An arcsine transformation was made to the total percentages of found MPs in different layers to ensure normality of the residuals. Statistical analyses were done with SPSS (version 23), and a one-way analysis of variance (One-way ANOVA) was applied to investigate the differences between numbers of microplastics in separate layers. The non-parametric Kruskal-Wallis test for independent samples was used to examine the number of ingested microplastics and the non-parametric Mann-Whitney U test was used to examine the distribution of different-sized microplastics in different sediment layers. The results are shown as average with standard deviation. Graphs were created using SigmaPlot 10.0.

3. Results

3.1. Grain size

The sediment was homogenous throughout the core of the examined unit. According to the classification by Blott and Pye (2001), the dominant fraction in each layer constituted of fine and very fine sand (250–63 μm) (50.6 ± 3.4%) followed by silt and clay (<63 μm), which made up 41.9 ± 3.6% of the sediment. Medium sand (500–250 μm) constituted 5.6 ± 0.4% of the sediment and coarse sand (> 500 μm) 2.0 ± 0.4%. The dry weight of all the sediment in one examined unit was 986.43 g.

3.2. Effects of time and bioturbation

Time (1, 2 or 3 week incubation) did not affect significantly the MP abundance below 1.7 cm when all units were grouped together, nor did it affect control units or animal units, when treated separately (Independent samples Kruskal-Wallis test, $p>0.05$). Hence the time was not taken into account in further analyses and all units were pooled.

The effects of bioturbation, including burrowing activity of the animals, was clearly seen as small holes on top of the sediment and as a lighter oxidized sediment layer reaching approximately the depth of 2.5 cm in all experimental units containing animals (Fig. 1). The oxygen concentration in all the cylinders during the experiment was 10.7 ± 1.2 mg/L when measured from the water phase of the units.

MPs were found throughout the sediment cores in both control and animal units. A clear vertical gradient in their distribution was observed: more than 90% of MPs were located on the top layer of the sediment (depth 0–1.7 cm) in all units and their abundance decreased towards the bottom (Fig. 2, Table 2). Animals significantly increased the abundance of MPs below 1.7 cm (One-way ANOVA, $p=0.000$). In control units 3.5 ± 1.2% of all MP particles were found deeper than 1.7 cm whereas in the units with animals 8 ± 2.7% of MPs were found in the deeper layers of sediment.

In the uppermost 5 cm of sediment (layers 1–3) the control units and animal units differed significantly from each other. The topmost layer (depth range 0–1.7 cm) contained fewer MPs in animal units compared to control units (One-way ANOVA, $p=0.000$) whereas the second (depth range 1.7–3.4 cm) and third layers (depth range 3.4–5.1 cm) had higher MP concentrations in animal units (One-way ANOVA, 2^{nd} layer $p=0.000$; 3^{rd} layer $p=0.010$). Below 5.1 cm (layers 4–6) the distribution of MPs was similar in all units ($p>0.05$).

Most of the animals were recovered from the sediment samples (Table 2). Of the 16 *M. balthica* individuals in each animal unit, on average 10.3 ±0.95 individuals survived until the end of the experiment. The location of all *M. balthica* individuals at the end of the experiment explained well ($p=0.000$, $R^2=0.81$, One-way ANOVA) the vertical distribution of MPs in the sediment. No significant effect was observed between the vertical distribution of MPs and *Marenzelleria* spp. ($p>0.05$, $R^2=0.03$, One-way ANOVA). The effect of *M. affinis* was not tested because they were all uniformly distributed in the topmost sediment layer.

3.3. Microplastic size

More microplastics of all sizes were found below 1.7 cm in the units with animals compared to control units (Independent samples Mann-Whitney U test; 50 µm, p=0.007; 150 µm, p=0.000; 300 µm, p=0.000) (Fig 3). A higher proportion of medium-sized MPs (150 µm) was found below 1.7 cm in both control and animal units compared to both larger and smaller particles. However, the overall abundance of different sized MPs was not statistically different below the first layer in control units and animal units (Independent samples Kruskal-Wallis test, $p>0.05$).

When comparing the effects of animals on the distribution of different sized MPs in different depths, animals increased significantly the concentration of all MPs in the second layer (1.7–3.4 cm) (Independent samples Mann-Whitney U test; 50 µm p=0.001; 150 µm p=0.000; 300 µm p=0.000) and the concentration of the largest MPs (300 µm) in the third (3.4–5.1 cm) and fourth layers (5.1–6.8 cm) (Independent samples Mann-Whitney U test; 3rd layer p=0.002; 4th layer p=0.019).

3.4. Ingested microplastics

Pieces of fishing line were ingested only by *M. balthica*. 19 individuals (25.3%) had ingested altogether 12 pieces of the smallest (50 μm), 25 pieces of the medium-sized (150 μm) and 24 pieces of the largest (300 μm) MPs. There was large intraspecific variation among the individuals that had ingested MPs: the highest observed concentration was 15 pieces in one clam, but on average the concentration was only 1.22 ± 1.06 pieces per animal. There were differences in the number of MPs ingested by clams at different time points: from the total of 61 ingested particles, 39 were found in the first week resulting in an average of 1.90 ± 1.16 pieces per individual, 9 in the second week (0.45 ± 0.25 pieces per individual) and 13 in the third week (1.30 ± 1.27 pieces per individual). However, these differences were not significant (independent samples Kruskal-Wallis test, $p>0.05$). Neither the size of the animal ($p>0.05$) nor the number of dead individuals in the same unit ($p>0.05$) explained the number of ingested MPs (independent samples Kruskal-Wallis test).

4. Discussion

It has been estimated that most particles that sink to the seafloor are displaced a few times by animals prior to their more or less permanent burial (Wheatcroft, 1992). In addition to natural particles, our results clearly demonstrate the ability of benthic animals to transport MPs deeper from the sediment surface. Compared to control units, a higher proportion of MPs was gradually distributed from the surface to a depth of 5.1 cm in the presence of animals indicating that bioturbation is an important process shaping the vertical distribution of MPs on the seafloor.

The intensity of bioturbation is dependent on the species composition due to the differences in specific characteristics such as feeding mode and typical burrowing depth (Viitasalo-Frösén et al., 2009; Josefson et al., 2012). Deposit-feeding has been suggested as being the most important

274 animal activity that affects particle displacement (Jumars and Wheatcroft, 1989), but particles can
275 also be displaced by animal movement in their preferred burrowing range. A positive correlation
276 between the MPs and animal distribution in the sediment was found only with *M. balthica*
277 indicating that bioturbation by the Baltic clam affects the vertical distribution of MPs. This is in line
278 with earlier laboratory studies showing that *M. balthica* is relatively efficient in displacing particles
279 vertically in the sediment (Viitasalo, 2007; Viitasalo-Frösén et al., 2009). Besides ingestion and
280 egestion, the particles above *M. balthica* could also fall into the space created around the clams
281 when they move around in the sediment (Viitasalo, 2007; Hedman et al., 2008). In our study it is
282 likely that both ingestion and movement in the sediment played a role in particle transport, because
283 MPs were also found inside *M. balthica.* This was expected because the species is known to ingest
284 natural particles of similar size (Gilbert, 1977; Viitasalo, 2007). However, *M. affinis* and
285 *Marenzelleria* spp. did not ingest any sized pieces of fishing line, which was most probably due to
286 the relatively large size of the MPs: *M. affinis* has not been observed to ingest particles >60 μm
287 (Ankar, 1977). Likewise, the MPs could have been outside of the preferred feeding range of
288 *Marenzelleria* spp., because individuals larger than the ones used in this study have been observed
289 to select smaller glass beads (88–127 μm) over larger ones (177–250 μm) (Bock and Miller, 1999).
290
291 The number of ingested MPs by *M. balthica* was relative to the number extracted from the
292 sediment, which is in accordance with an earlier observation that the number of ingested MPs by *M.*
293 *balthica* is related to the offered concentration (Setälä et al., 2016b). MP concentration in the
294 environment was also found to correlate positively with the number of MPs in the gut contents of
295 another non-selective deposit feeder, the lugworm *Arenicola marina*, which was exposed to
296 polystyrene particles in laboratory conditions (Besseling et al., 2013). That study also showed that
297 MPs within the feeding range of *A. marina* were not accumulated inside the animals but were
298 egested. A similar observation was made in our study with *M. balthica*, where the highest number

of ingested MPs was found on the first sampling occasion, indicating that the plastic particles were not accumulating in clams during the experiment. However, the particles used in this study were quite compact in shape; it is thus possible that fibrous or more irregularly shaped secondary MPs might behave differently inside the digestive tract (e.g. Murray and Cowie, 2011).

Although a fourth of the examined clams ingested MPs during the experiment, the average number of ingested particles per individual was relatively small despite high experimental concentration. When considering the sediment volume in each unit, we used a concentration that was several orders of magnitude higher than many studies have detected in nature (Claessens et al., 2011; Fischer et al., 2015; Frias et al., 2016). Nevertheless, it can still be considered environmentally relevant on the higher limit: for example, concentrations of similar magnitude have been found in sediments in the Lagoon of Venice, Italy, where the <1 mm MP concentration ranged from 672 to 2175 particles/kg (dry weight) (Vianello et al., 2013). Similarly, 3600 MPs/kg (d.w.) >38 µm by their size have been reported in the Nyborg Fjord in Danish straits (Strand et al., 2013), and 3320 plastic spheres/L in the subtidal sediments of an industrial harbour of Stenungsund, Sweden (Norén, 2007).

The number of ingested MPs found in the clams at each sampling occasion represents a snapshot of the 3-week incubation period, thus reflecting a continuous process of ingestion and egestion of the particles. The large variation in the number of ingested particles between individuals may be due to the feeding habits. *M. balthica* is a facultative deposit- and suspension-feeder that lives inside the sediment and extends its incurrent siphon to the sediment surface or overlying water to feed on organic matter (Self and Jumars, 1988; Lin and Hines, 1994). The average size of *M. balthica* in our study was 17.3 mm, and this sized clams typically have a feeding area diameter of less than 2 cm (Zwarts et al., 1994). In environmental conditions with resource competition and unfavourable food

conditions they tend to locate themselves shallower in the sediment and feed on organic matter within the sediment instead of filtering the water above the sediment surface (Lin and Hines, 1994). This may have been the case in our study, since during the experiment the units were not receiving any additional food apart from the organic material coming with the dripping seawater, and at the end of the experiment 97.5% of *M. balthica* individuals were located near the surface, in the topmost 3.4 cm of sediment. The high variation in the numbers of ingested MPs may thus be due to food scarcity and MP patchiness on the sediment surface (Setälä et al., 2016b). Additionally, in the beginning of the experiment some of the pieces of fishing line formed aggregates and it is possible that they were initially unevenly distributed.

Our results did not show significant differences in the total abundance of different sized MPs below 1.7 cm in the animal units, but the largest particles (300 µm) were more abundant in the depth of 3.4–6.8 cm compared to other size classes. Because *M. baltica* was rarely found in these depths in our study, it is possible that *Marenzelleria* spp. have influenced the MP distribution inside the sediment where they were the most abundant. *Marenzelleria* spp. are surface and subsurface deposit-feeders, that are known to move particles in random directions when feeding and maintaining burrows (Quintana et al., 2007; Norkko et al., 2012). Because *Marenzelleria* spp. was not observed to ingest any MPs, they may have aided the transfer by burrowing activities. This effect was seen only in the case of larger particles, which may have been due to a higher probability to encounter larger particles compared to smaller ones even if their concentrations were similar (Jumars et al., 1982). It has also been suggested that coarser particles would be more easily transported downward because their gravity overcomes the cohesive and adhesive forces between sediment grains (Wheatcroft, 1992). However, because we used relatively low densities of *Marenzelleria* spp., it is difficult to verify their influence on MP distribution. Nevertheless, based on our results benthic communities are capable of shaping the vertical distribution of MPs and

because *M. balthica*-dominated communities similar to this study are common in the Baltic Sea (Josefson et al., 2012), such assemblages may have important implications in natural sediments.

In some parts of the Baltic Sea, such as in the Gulf of Finland, the benthic communities are dominated by *Marenzelleria* spp. rather than *M. balthica* (Josefson et al., 2012). The densities of *Marenzelleria* spp. used in our study were relatively low compared to the highest densities (over 5 000 individuals/m^2) found on the soft bottoms of the Gulf of Finland (Rousi et al., 2013; Kauppi et al., 2015). Burrows of *Marenzelleria* spp. can penetrate up to 30 cm into the sediment (Hedman et al., 2008; Norkko et al., 2012), and therefore might distribute MPs even deeper than what was observed in this study. As bioturbation intensifies with population density (Adámek and Maršálek, 2013), the influence of *Marenzelleria* spp. on MP distribution might be observable in higher population densities. *Monoporeia affinis*, on the other hand, is known to inhabit only the top few centimeters of sediment (Viitasalo-Frösén et al., 2009). Even though its activity on the upper layer of the sediment is known to increase particle resuspension in the water (Hedman et al., 2008), its effects were not detectable in this study because its penetration depth was mostly within the first sediment layer.

Our study lasted for three weeks, which was probably not sufficient to detect the effect of time on MP distribution. However, evidence of time influencing the vertical distribution of luminophores in sediments inhabited by *Marenzelleria viridis* has been observed by Quintana et al. (2007) who found that marked changes in the vertical distribution of luminophores (e.g. 99% penetration depth increasing from 2.7 cm to 5.7 cm) took place between the 37^{th} and 51^{st} research days. Likewise, a longer incubation time could have shown differences in the transportation of different size classes of MPs. Taking into account the longevity of plastics in the marine environment and the local

bioturbation activity of benthic invertebrates throughout their lifespan, the distribution patterns of MPs should be monitored on a much longer timescale than what was used in this study.

So far studies investigating MP abundances on the seafloor have sampled variable depths of sediment ranging from 1 to 5 cm (Van Cauwenberghe et al., 2013; Vianello et al., 2013; Woodall et al., 2014; Talvitie et al., 2015). This study suggests that a proportion of MPs can be distributed to 5 cm depth in just three weeks due to the activities of common infauna in the Baltic Sea. However, the intensity and depth of bioturbation depend on the local conditions and characteristics of the benthic community. An estimated worldwide mean depth for bioturbation is nearly 10 cm (Boudreau, 1998), so MPs in the natural sediments may be present at even greater depths than what was observed in our study or that was sampled in previous field studies. Evidence on vertical distribution of MPs in subtidal sediments is still missing, but earlier investigations have demonstrated that microplastic abundance decreases with depth in beach sediments (Carson et al., 2011; Turra et al., 2014). Interestingly, most of the plastic pellets were distributed below the most often sampled topmost 5 cm of the beach sediment, and some were found as deep as 2 m (Turra et al., 2014). These sediment studies together with our study indicate that taking sediment samples from only the top 1–2 cm may fail to represent the true abundance of MPs in the sediments, but serve merely as an indicator for the microplastic load to the environment.

As particles sinking from the water column to the seafloor are slowly buried by bioturbation they may end up in the layers where hazardous substances, such as polychlorinated biphenyls (PCBs), are known to exist (Konat and Kowalewska, 2001). There is also evidence that MPs tend to accumulate in areas with low hydrodynamics alongside finer sediments and associated contaminants (Vianello et al., 2013). Since it has been observed that plastics are able to adsorb many persistent organic pollutants (POPs) from the surrounding seawater (Frias et al., 2010; Rios

et al., 2010) and sediments (Ghosh et al., 2014), they may open a new pathway for these contaminants to enter marine food webs. Laboratory studies have indicated that MPs in sediments can either increase or decrease the bioaccumulation of POPs in animals (Besseling et al., 2013; Koelmans et al., 2013b, a), but it is not yet known if the exposure to POPs via MPs in the nature is substantial compared to other sources. It is also unclear whether MPs are permanently buried in the marine sediments, or are able to re-enter the food webs if released back to the sediment-water interface due to bioturbation or dredging.

5. Conclusions

To our current knowledge, this is the first study to experimentally demonstrate the vertical distribution of secondary MPs in soft marine sediments and the effect of bioturbation by benthic invertebrates on their transportation. Vertical transport of MPs has recently been defined as one of the research priorities regarding the distribution and fate of MPs in the marine environment (GESAMP, 2016), and based on our results it can be concluded that bioturbation plays an important role in transferring MPs deeper within the sediment. As a consequence, this might eventually lead to the burial of MPs and thereby decrease their availability to organisms feeding on the sediment surface. The rather rapid burial of MPs observed in this study also supports the idea of the seafloor being an ultimate sink for marine microplastics (Woodall et al., 2014). However, if MPs are not permanently buried in the seafloor, their possibly overlapping distributions with hazardous substances in the sediment may be an emerging threat to food webs. This study highlights the need to gain additional knowledge about the interactions of MPs with benthic fauna and hazardous substances to better assess their fate in our oceans, especially when the amount of MPs is estimated to rise in the marine environment (Thompson, 2015). Furthermore, our results imply that sediment

samples for monitoring purposes should include sediment below the thin surface layer to get a reliable picture about the MP reservoirs on the seafloor.

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Table 1. Mean sizes, abundances and densities of individuals added to the animal units. Natural abundances are based on the data of Rousi et al. (2013) taken from the depth of 35 m during years 1993–2007.

	Macoma balthica	*Monoporeia affinis*	*Marenzelleria* spp.
Mean size (mm)	17.3 (SD±1.4)	not measured	20*
Individuals per unit	16	3	8
Abundance per unit (m^2)	1038	195	519
Natural abundance (m^2)	200–1100	30–800	8–7000

* estimate, could not been measured due to fragmentation during the preservation

Table 2. Average percentages of MPs with standard deviations found in different depths of sediment of control and animal units and the distribution of study animals at the end of the experiment.

		MPs control	MPs animal	*Monoporeia affinis*		*Macoma balthica*		*Marenzelleria* spp.	
Layer	Depth (cm)	%	%	ind.	%	ind.	%	ind.	%
1	0-1.7	96.5 ±1.2	92.0 ±2.7	33	100.0	144.5*	60.2	11	11.5
2	1.7-3.4	1.2 ±0.8	4.7 ±1.6	0	0	89.5	37.3	23	24.0
3	3.4-5.1	0.8 ±0.6	1.4 ±0.8	0	0	4	1.7	31	32.3
4	5.1-6.8	0.5 ±0.3	0.7 ±0.4	0	0	1	0.4	22	22.9
5	6.8-8.5	0.5 ±0.3	0.4 ±0.3	0	0	1	0.4	3	3.1
6	8.5-10	0.5 ±0.5	0.8 ±0.7	0	0	0	0	6	6.3
End total				33		240		96	
Start total				45		240		120	

*one clam was cut in half when slicing sediment layers 1 and 2

Figure 1. Experimental cylinders at the end of the experiment; control unit (left) and unit containing animals (right). The oxidized layer is clearly visible on top of the sediment core in the animal unit, seen as lighter grey layer.

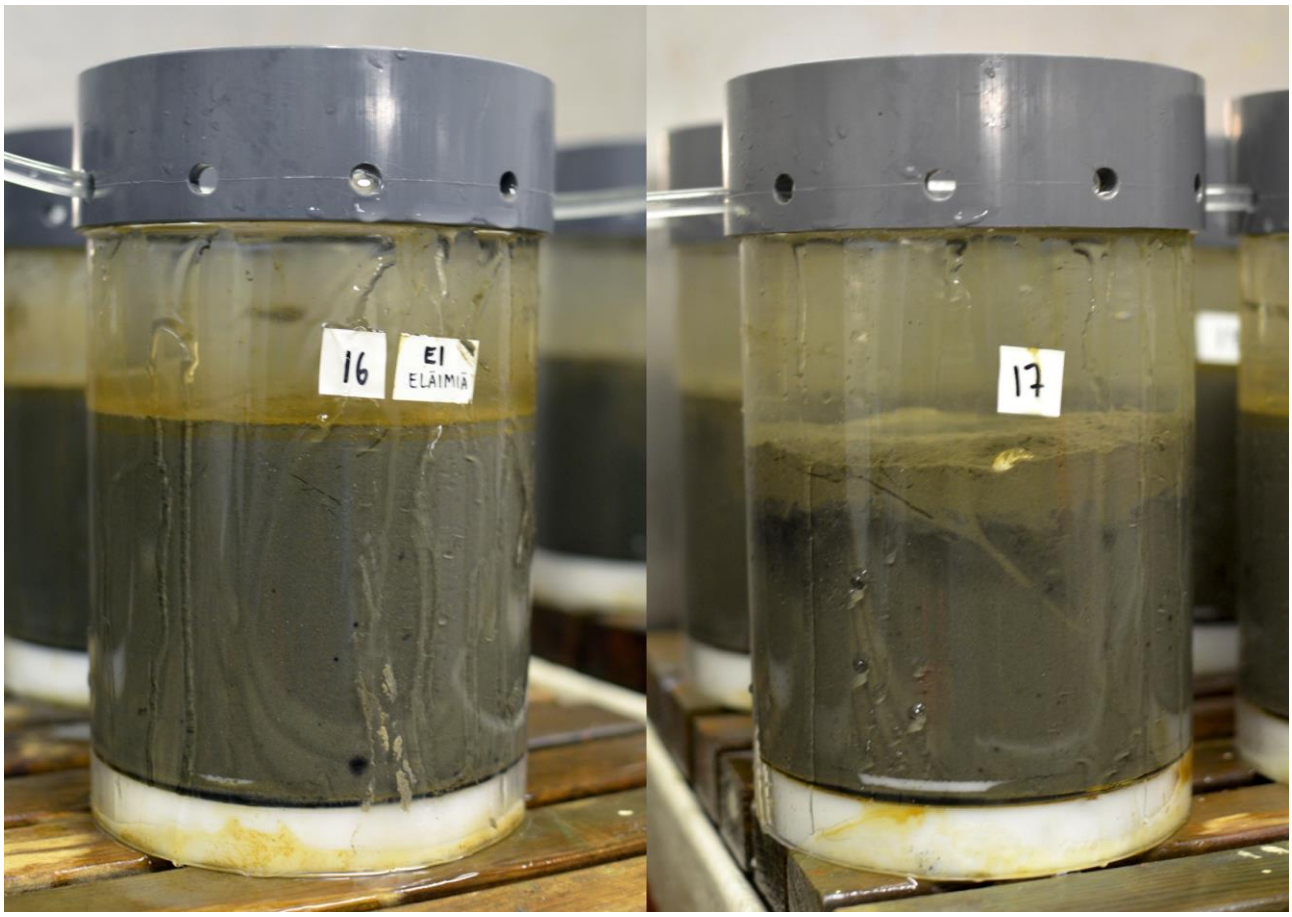
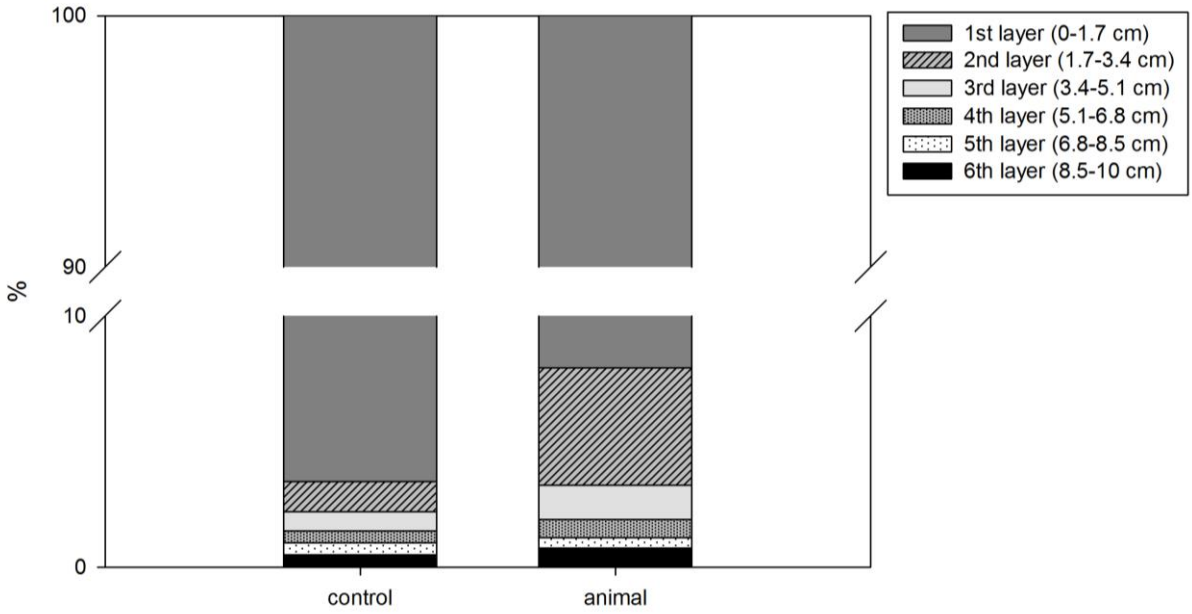


Figure 2. Mean abundance of MPs in different sediment layers of control and animal units.



681 Figure 3. Average abundances and standard deviations of different sized MPs found below the
682 depth of 1.7 cm in both control and animal units.
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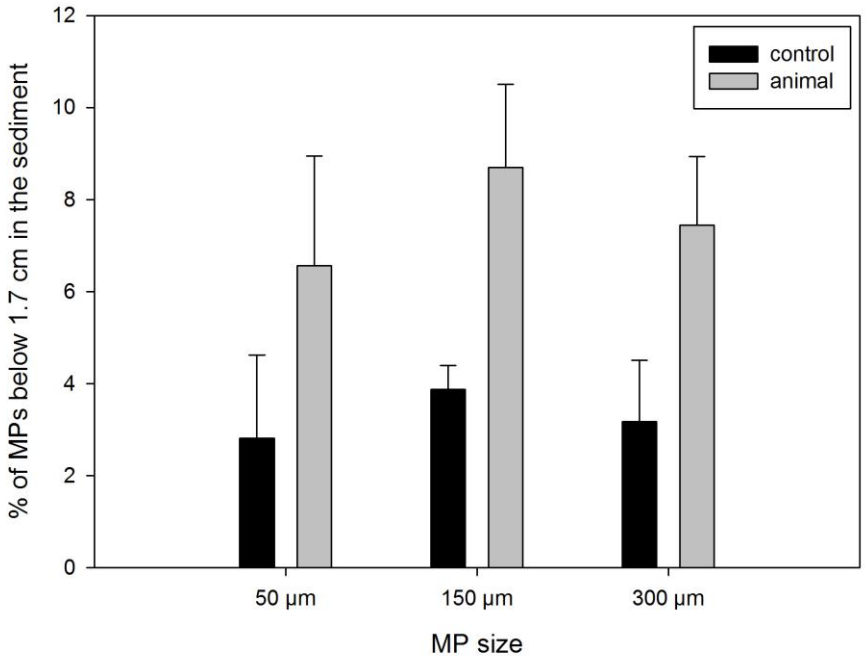

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